Citrus Health Research Forum – Denver, Colorado
June 16-18, 2010

COMPILED REPORT FROM OUTCOME 2 BREAKOUT GROUP

OUTCOME 2: Slow/Stop Spread of HLB

June 17, 2010 afternoon session:

Participants discussed the primary components of a system needed to slow or stall the spread of citrus greening: improving understanding of ACP behavior and disease transmission, improving detection and monitoring of the vector, and pursuing control and management options. In particularly, discussions focused significant attention on current understanding of the vector and important gaps in knowledge, including its behavioral dynamics and how they can be modified, as well as various biological, chemical, and cultural pest management and control strategies, and how they can be integrated into overall best practices and an Areawide control program.

As part of these discussions, participants emphasized the need to test the various hypotheses in different geographical regions because there are specific factors that affect the best management tactics for each region. Also, because non-crop areas vary with geographic region, outreach efforts may need to be tailored to each regional audience. Industry can leverage funds to make sure the research is accomplished in all regions.

Also important is the need to involve the citrus industry and neighboring countries—particularly Mexico and the rest of Central America—in the research and extension efforts.

Output 1. Knowledge of ACP behavior/ Liberibacter interaction with ACP

Topic 1: Biology of transmission Current research and questions:

- What determines successful transmission?
- Rates of transmission are 1-2 percent. Lab colony may be weak.
- Look at the microenvironment of trees (humidity in the canopy), pruning may change that. Florida: collected nymphs and always positive, but the plants don't have symptoms. Brazil: adults can acquire from asymptomatic plants (60 days), with very low infection levels.
- Psyllid can have high titers in salivary glands but doesn't transmit so maybe it is missing a symbiont? Orlando 2008 researchers had different interpretations of their results and the thresholds of titer that were significant. Influence of temperature on the transmission ability of ACP. Transmission rates are so low it is difficult to test. Titer in salivary glands is very high with little variation.
- Varietal and rootstock effects: some are more susceptible than others.
- Use nymphs for early detection of low titer? Probably not faster than using leaf samples.
- Helper component for liberibacter? Phytoplasma reports – still not proven. Seldom are the bacteria and phytoplasma found together. Researchers have found other organisms

associated with the disease, that don't cause the disease, but affect the immune system of the plant. It takes the interaction between the pathogens to cause disease. Multipathogen system. There could be 3-4 organisms silencing genes. Some plants loaded with liberibacter are doing fine. Symptomology takes 6 months to 1 year. The psyllid also interacts with the plant and bacterium.

- Short-term strategies and long-term strategies are needed.
- African psyllid and africanus to provide information about this system: response of host plant.
- If the Psyllid can acquire from an asymptomatic plant then taking out the symptomatic plants doesn't help. So the vector transmission information does help with management strategies.

Output Needs: plant physiologist + genomics + bacteriologist + vector biologist team and a clean culture of the bacterium!

Topic 2: Behavior modifiers (attractants and repellents) Research questions as follows:

- Is there any host plant that is very attractive that could be used as a trap crop? *Murraya paniculata* is a favored host plant in Florida. Our environment is synthetic. We don't know the cues in the original habitat.
- Could repellents used to move ACP to trap crops?
- Use an attract and kill strategy in urban or other areas.

Topic 3: Effect of environment on Liberibacter. Research that is currently being conducted or is needed:

- Arizona desert: look at temperature limits for HLB multiplication in citrus. Arizona is more extreme than Brazil. In Pakistan where temperatures are extreme, they prune and the disease rises and falls with temperature. What happens to the titer and does the disease move to the roots when temperatures are extreme?
- Brazil las and lam: lam likes temperatures below 30°C, whereas las can tolerate higher temperatures. NE Brazil is dry and hot and see fewer ACP and no HLB.
- Lee and Roose have a project with Pakistan, monitoring varieties for ACP feeding and HLB incidence to get this type of information.
- China: areas where temperatures are more extreme seem to suppress psyllid and disease.
- Texas: ¾ of citrus is 25-35 days > 38°C and the temperature is in Texas favor. This may be why Texas does not have the disease.
- Perhaps add antibiotics to the situation.
- Experiments are designed to look at titers that are very low and there is a lot of variation in interpretation of titer results, how the experiments are set up. Need standardization or clarity in how the assays are done and interpreted.

Topic 4: Study specific haplotypes

Deliverables: Behavior model (Mizzell et al. 2008 Ann Rev Entomol.): Knowledge of ACP behavior with and without Liberibacter, new attractants and repellents, new management strategies based on the environment (physical and biological).

Milestones: Measured vector capacity/efficiency and stability over time.

Extension: Translated into terms that the urban and grower communities can understand. Measure understanding of the biology and behavior of ACP and HLB.

Output 2. Psyllid detection/monitoring (tap, sweep, sticky card):

Review what research has been completed and draw inferences. Standardize methods of monitoring to better compare regions. Fill in gaps in methods. Share information between states. Research conducted and identified gaps are included in topics for Output 4.

Topic 1: Improve the ability to detect or monitor ACP

Topic 2: Relative effectiveness/efficiency of various monitoring devices across all states related to the desired objective

Topic 3: Recognize that there is a lag between ACP arrival and detection

Topic 4: Trap in relation to presence of flush, irrigation and other factors

Topic 5: How does trap density relate to the population density?

Topic 6: Detect HLB in the psyllid: Validate what the results mean at different times of the year and different environments (detection efficiency), use laboratory resources wisely, have confidence in results. Collaborate and coordinate with Outcome group 1

Deliverables: more effective detection or monitoring systems, new attractants, evaluate traps in different locales, develop trapping protocols for growers and agencies, develop a formula to relate the number of ACP in traps to the population density, develop sampling programs.

Milestone: Development of programs and validation of programs.

Outreach and Extension: Extend sampling programs to each audience using the appropriate communication methods or tools. Measure understanding and adoption of techniques.

Output 3. Mitigation of new introductions of ACP (included under Output 4)

Output 4. Area-wide management of established ACP infestations

Topic 1: Define what we know about areawide management and share the information

Topic 2: Identification of landscape features (influence of abandoned orchards, alternate ACP hosts and dooryards) –define the level of risk these areas have for commercial citrus.

- Texas: Mexican fruit fly traps collecting ACP in urban and production areas. In the pilot area-wide program in Texas, sampling both situations. These will trace

movement or at least suggest a pathway. Once the big grove is cleaned up with treatments, should see the influence of dooryards.

- Arizona and California: surveying dooryards but not doing studies – eradication is occurring.
- How many are moving back and forth? Mark and recapture studies are needed. If there is areawide management of orchards, but dooryards are not treated, what is the impact? Brazil: not concerned about dooryard because the production areas are so much larger. It becomes an issue if you are in an eradication program. At some point in time the program changes from eradication to suppression and need the movement information to decide what level of movement is acceptable. (Eradicate = maximum suppression. We don't eradicate, we suppress the insects. The word eradication is used for trade and regulatory purposes. The term eradication is used to describe localized extinction and don't find the organism for a set period of time.) Area-wide management: we need to know how many come out of unmanaged situations. Abandoned orchards are a similar problem (dooryard = unmanaged = abandoned). Both sources of psyllids but in terms of regulations, abandoned orchards can be required to spray, and dooryards have different regulations. It may be possible to apply milk spray to dooryard trees and see if psyllids appear in the commercial orchards. Populations in dooryards depend on the homeowner. Abandoned orchards are lack of maintenance and more predictable in their effect. In Arizona, abandoned orchards die due to water issues. Need interaction and education of the homeowners to get them involved in the areawide treatment programs. This gets into outreach. Dooryards will always be a source of psyllids and/or HLB. Is it a big enough risk to fight the battle of treatments and tree removal. It is a gap in our knowledge. Texas abandoned grove has lower populations of ACP in abandoned groves. Especially in dry years.

- Output: How would you do the study? Mark, release, recapture. With milk and other markers, could have multiple treatments to measure movement in both directions. Intensive yellow card trapping in treated orchards to monitor border influences. Molecular markers are a possibility. Symbionic variations. Need to know when, where and how it moves. The nature of the host could influence the movement. Need thousands of ACP to do the studies to get the recapture.

- Risk analysis needs to be done in both managed and unmanaged groves or areas.

<u>Topic 3: Develop an integrated pest management approach (biocontrol + chemicals + semiochemicals, cultural) to ACP management</u>

- Data needed to establish a management plan: what do the ecosystems contribute to the problem (dooryard, commercial, nursery etc)? What management tools do I have (chemical, bc)? Can I get all the affected groups to cooperate? How big of an area?
- There is much more than just outreach to homeowners. Everyone has to work together at the same time and it can't be done without significant funding.

- If we are going to get cooperation of homeowners, must have money for education outreach and treatments.
    - It may be possible to treat the borders rather than the whole orchard.
- We need many more management tools for urban, commercial etc.
- Define the areawide pest management program – control of psyllid. With Florida, there is disease as well and variation in incidence of the disease. Need funding from growers, government, and public sector. Build on the strengths of the partners. Growers participate in monitoring efforts. APHIS can turn data around to help growers make management decisions.
- **Areawide discussion needs its own working group.**

Topic 4: Determine thresholds of ACP and HLB incidence that generate control tactics
Topic 5: How big is an effective area wide program? (need to establish parameters)

Texas: 5,000 acres in the pilot project. The beginning stages. No funding to do dooryard treatments. If funding were available, this would be done. Balance trapping and monitoring with treatment. If you don't treat, what is the point of the trapping? The size of the area is based on funding. Citrus is fractured and chose an area where the groves are bigger and more contiguous to utilize aerial applications.

Florida: Grower associations determine the size of the treatment areas.

Brazil: areas that are contiguous and larger growers are being treated with aircraft.

i. Dependent on distribution of the psyllid
ii. Dependent on ecosystems involved
iii. Wind patterns
iv. Presence of HLB

Topic 6: Population dynamics of ACP (foundational research)

i. Psyllid movement (triggers of movement, geospatial modeling, routes of invasion, introduction frequency, regulatory consequences)

- Flying away from citrus during the season? Routinely fly 100-200 meters away, especially in the spring. Looking for mates, food, or oviposition sites which is trivial flight. Then there is migratory flight when the food and mating is ignored. We don't know if ACP ever does migratory flight. Some psyllids are attracted to plants in the cage, some want to leave.
- How can we document migratory movement? Airplane collections, put sentinel plants in Georgia, in Texas where there is desert. Flight muscle and reproductive state might demonstrate migratory versus trivial flight.
- Why are some trees more attractive? Why are there party trees?
- How far do they fly?
- Controlled studies could be done to use Mexican lime trees in dooryards and expose them to different levels of drought or pruning to measure movement.

Sociological issues: Mexican limes flush after fruit is picked and homeowners pick fruit unripe.

ii. Psyllid behavior includes the following topics:

- How does the psyllid select a host plant? What is its host range? How does the psyllid know a plant is a host plant? Florida has a grant with China to screen all of the USDA-ARS genome for which varieties have resistance to the psyllid, which varieties serve as food hosts and which serve as reproductive hosts.
- Are psyllids observed on non-Rutaceous hosts? They will survive on other hosts for 7-8 days (cotton, grapes). Texas has reports of ACP living on and moving from potato fields. Huge populations in Texas on every type of host. Potato psyllids do well on citrus, but it seems to be more polyphagous. Brazil found that during mild winters, ACP can survive 7-8 days without feeding.
- Will ACP feed on artificial diets? The cues don't have to have citrus chemicals. There may be signaling once they get inside the phloem. The sugar water has to be green to attract them. Love ridges in the parafilm.
- Edge effect: more light on the edge of the grove. Trapping on the edge has been done in Florida. SE corner has the most ACP and there is a minor peak in the NW corner. Texas has visual observations that there was significant decrease after the first 5 rows. Texas sees a SE quadrant effect of higher numbers. Hard to say it is wind (Texas prevailing winds off of the Gulf). SE corner is the most benign part of the tree (warm in morning, cool in afternoon). True of other pests such as glassywinged sharpshooter in California. Could target treatments to that corner. Highly phototaxic, morning sun. Will fly toward the sun in the morning.
- Time of day for flight activity: don't disperse at night. Texas 3-4 hours 10 am-1pm. Optimize time to spray.
- What is a border? Irrigation ditch, break in trees, roads.
- What makes and ACP avoid plants? Repellents. Move them to a trap crop. Attractants could be used as attract and kill in urban areas.

<u>Topic 7: Best Management Practices Guidelines for each area (constraints of irrigation, spray schedule, and integration with natural enemies)</u>

<u>Topic 8: Alternatives to chemical treatments in areas where you can't treat and for long-term solutions</u>

<u>Topic 9: Resistance management</u>

<u>Topic 10: Impacts of the components of management (vector control + tree removal) on the success of the program</u>

- Can HLB be eradicated or slowed by controlling the vector? Florida: Have to be willing to do control for 2-3 years with infected-tree removal. China: There are areas in China where the disease is controlled with insecticides. Block plantings

are needed; individual tree replacement doesn't work due to surrounding infected trees.

- Florida growers that allowed infection to continue and used nutritionals, assumed that controlling ACP eliminated their HLB threats to their neighbors.
- IF HLB is found in Texas or California can it be eradicated? Depends on the size of the infection which is why early detection and delimitation is critical.
- Glassy winged sharpshooter experience, it was chemically intensive at first, but changed to 5-year cycle of treatment with intensive monitoring. Hopefully we can move to that strategy.
- Florida populations have declined. Intensive management during 2005-2008. Last 2 years have shifted to dormant treatments with soft chemistries during the season.

Deliverables: Executable plans for the various regions, models, economic analysis, GIS mapping of citrus integrated with ACP populations, treatments, and other factors, more tools (biocontrol agents, chemicals, cultural practices), pesticide rotation strategy (stewardship protocol), best management practices, strategies for responding to different levels of noncompliance, and engagement of the community

Milestones: measuring the increased participation of the users (citrus, urban etc).

Outreach and extension: Educating users with the cost effectiveness of the plan. Need a high proportion of funding for education and outreach in management programs. Every user needs to be engaged to make the program effective.